Arabidopsis Species Hybrids in the Study of Species Differences and Evolution of Amphiploidy in Plants[1]

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It is estimated that 5 million years of evolution separate *Arabidopsis thaliana* from its close relative *Arabidopsis lyrata.* The two taxa differ by many characteristics, and together they exemplify the differentiation of angiosperms into self-fertilizing and cross-fertilizing species as well as annual and perennial species. Despite their disparate life histories, the two species can be crossed to produce viable and vigorous hybrids exhibiting heterotic effects. Although pollen sterile, the hybrids produce viable ovules and were used as female parent in backcrosses to both parental species. The resulting backcross plants exhibited transgressive variation for a number of interesting developmental and growth traits as well as negative nuclear/cytoplasmic interactions. Moreover, the genesis of a fertile amphidiploid neospecies, apparently by spontaneous somatic doubling in an interspecific hybrid, was observed in the laboratory. The mechanisms responsible for the generation of amphiploids and the subsequent evolution of amphiploid genomes can now be studied through direct observation using the large arsenal of molecular tools available for Arabidopsis.

Plant growth and development have traditionally been studied by generating relevant mutations or by analyzing naturally occurring variants within a species. In only a few cases has the tremendous interspecies variation that was generated over the millions of years of evolution been used. In recent years, it has been increasingly recognized that natural variability is a major resource that could complement traditional approaches. Thus, in the model plant Arabidopsis, intraspecific genetic variation has been noted among different geographical isolates, and this variation, which is largely quantitative in nature, is being subjected to analytical methods developed for the analysis of quantitative trait loci in crop plants (for review, see Alonso-Blanco and Koornneef, 2000). However, the enormous store of natural variation that is manifest in interspecies differences has remained largely untapped.

Wide crosses and interspecific hybridizations have been used to investigate the genetic basis of complex traits that differentiate varieties within a species as well as related species in several plant families (Doebley et al., 1990; Bernatzky et al., 1995; Bradshaw et al., 1995; Bernacchi and Tanksley, 1997; Eubanks, 1997; Lin and Ritland, 1997;). The development of an interspecific hybrid model would be particularly useful in the genus Arabidopsis. The availability of the Arabidopsis genome sequence in public databases provides unique opportunities to re-examine concepts of speciation and to understand in molecular detail some of the factors associated with species diversification. The generation and analysis of interspecific hybrids between *Arabidopsis thaliana* and related species would also provide an additional resource for the functional analysis of the Arabidopsis genome.

The feasibility of generating interspecific hybrids of Arabidopsis and closely related species is suggested by the occurrence of *Arabidopsis suecica*, an allotetraploid thought to be derived from *A. thaliana* and *Cardaminopsis arenosa* (Hylander, 1957; Mummenhoff and Hurka, 1995; O'Kane et al., 1996), which occurs naturally and can be synthesized in the laboratory by crossing autotetraploid *A. thaliana* (generated by colchicine treatment) and tetraploid *C. arenosa* (Chen et al., 1998). In the 1950s to 1970s (Laibach, 1958; Berger, 1966; Redei, 1972, 1974), interspecific hybridizations were performed in an attempt to clarify the taxonomic relationships of *A. thaliana* to related species. Laibach (1958) performed crosses between *A. thaliana* and the allotetraploid *Cardaminopsis suecica* (now *A. suecica*) and produced, after ovule rescue, sterile F_1 hybrids. Berger (1966) subsequently succeeded at producing seed by crossing *A. thaliana* and polyploid *Arabidopsis pumila* ($2n = 32$), and Redei (1972, 1974) obtained viable seed and fairly fertile F_1 hybrids by crossing *A. thaliana* with tetraploid *C. arenosa* ($2n = 32$). Hybridizations of *A. thaliana* with related diploid species were rarely performed, although both Mesicek (1967; quoted in Redei, 1972) and Redei (1974) crossed *A. thaliana* with *Cardaminopsis petraea* ($2n = 16$) each raising a sterile hybrid plant ($2n = 13$) that was not characterized further. To our knowledge, however, crosses between diploid species in the genus Arabidopsis have not been used either to uncover naturally occurring variation or to construct stocks for genetic analysis of traits that differentiate species within the genus.

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For interspecific hybridization studies in Arabidopsis, we elected to use *A. thaliana* and *Arabidopsis lyrata* because this species pair and their hybrids present several advantageous attributes for the study of interspecific variation. First, molecular systematic analysis indicates that the two species are closely related and diverged from one another 3.8 to 5.8 million years ago (Kuittinen and Aguade, 2000). Second, the genomes of the two species share a high degree of sequence similarity that allows facile transfer of molecular markers and other data generated by the Arabidopsis genome project to *A. lyrata* (van Treuren et al., 1997; see below). Third, differences in chromosome numbers between the two diploid species (*A. thaliana* is $2n = 10$ and *A. lyrata* is $2n = 16$ [van Treuren et al., 1997]) indicate that genetic divergence has significantly altered the basic genetic apparatus of the two species. Fourth, *A. lyrata* is self-incompatible and therefore it is by and large an outbreeding species in contrast with *A. thaliana* that is a self-fertilizing species that rarely outbreeds. Finally, from a developmental standpoint, the two species differ for a variety of morphological traits. These include quantitative differences such as larger mass of floral organs, fruit, and seed in *A. lyrata* relative to *A. thaliana*. The two species also exhibit qualitative differences for various traits, including leaf morphology, the tendency of *A. lyrata* but not *A. thaliana* to produce aerial rosettes, as well as an annual and ephemeral existence in *A. thaliana* in contrast to a perennial growth habit in *A. lyrata*.

In this paper, we describe the production of hybrids between *A. thaliana* and *A. lyrata* (formerly *Arabis lyrata*), a species that has been recently incorporated into the genus Arabidopsis on the basis of molecular data (O'Kane and Al-Shehbaz, 1997). The properties of the hybrids, of progenies resulting from backcrosses to the parental species, and of a spontaneously generated amphiploid are described.

RESULTS

Generation and Analysis of Arabidopsis Interspecific Hybrids

To generate interspecific hybrids between *A. thaliana* and *A. lyrata*, we first carried out pollination tests to determine whether to use *A. lyrata* or *A. thaliana* as the female parent. Microscopic analysis of pollinated flowers revealed that the *A. thaliana* stigma epidermis supports efficient adhesion, hydration, tube emergence, and growth of *A. lyrata* pollen, and these interspecific pollinations resulted in the development of viable seed from which mature plants could be generated by ovule rescue ("Materials and Methods"). In contrast, the reciprocal cross was not as productive, possibly due to cross incompatibility. Several *A. thaliana* × *A. lyrata* crosses were made by removal of the un-dehisced anthers from *A. thaliana* flowers and manual transfer of *A. lyrata* pollen. Five progeny plants were grown and confirmed to be true interspecific hybrids by cytological analysis, which showed the presence of 13 chromosomes (i.e. the sum of the basic chromosome number of *A. thaliana* [$n = 5$] and that of *A. lyrata* [$n = 8$]; Fig. 1). In addition, DNA gel-blot analysis demonstrated the inheritance of restriction fragments from both parental species (Fig. 2).

The hybrid status of the progeny plants derived from these crosses was also evident from a variety of morphological characteristics, as illustrated in Figure 3, for the size and arrangement of petals. *A. lyrata* petals are approximately 30 times larger than *A. thaliana* petals and are arranged in an X-pattern rather than the cruciform pattern typical of *A. thaliana* flowers. In the hybrids, the petals were smaller than *A. lyrata* petals but still an order of magnitude larger than *A. thaliana* petals, and their arrangement was also intermediate between the two parental species (Fig. 3). However, several growth characteristics were similar to one or the other parent. For example, the hybrids were similar to their *A. lyrata* parent with respect to plant stature and production of aerial rosettes. Also like their *A. lyrata* parent, the hybrids required an extended vernalization period for flowering and were long lived. For still other traits, the phenotype of the hybrids exceeded that of either parent (i.e. they exhibited transgression); for example, stigma size was larger in the hybrids than in the parents. It is interesting that, as often observed in interspecific hybrids, the *thaliana-lyrata* hybrids exhibited hybrid vigor. This vigor was evident in the increased rosette and root mass of hybrid seedlings relative to the parental species (Fig. 4). In mature plants, hybrid luxuriance was manifested by increased numbers of inflorescences and flowers (Fig. 4).

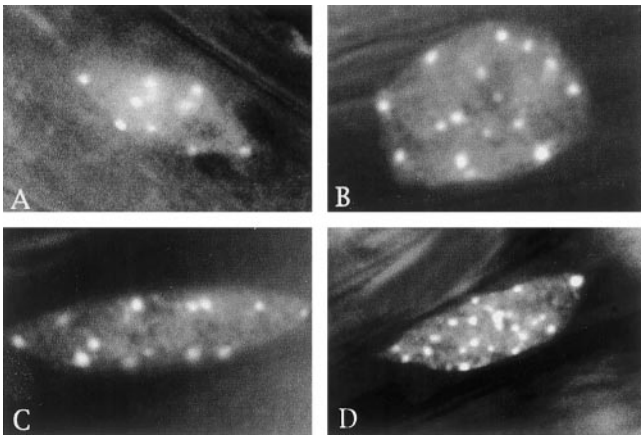

Figure 1. Chromosome counts of the *A. thaliana* (A) and *A. lyrata* (B) parental species, their F_1 hybrid (C), and derived amphidiploid (D). Chromosomes were visualized by 4′,6-diamino-phenylindole staining.

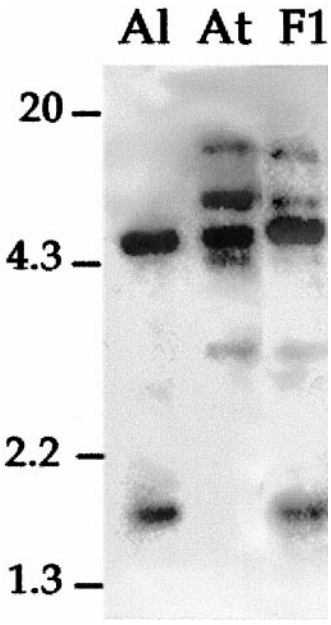

Figure 2. DNA gel-blot analysis of the *A. lyrata* (Al) and *A. thaliana* (At) parental species and their F_1 hybrid. DNA was digested with *Hin*dIII, and the blot was hybridized with a probe derived from the *AtS1* gene of Arabidopsis (Dwyer et al., 1992). Molecular size standards in kilobases are shown to the left.

Variation for Developmental Traits and Chromosome Assortment in Backcross Progenies

The *thaliana-lyrata* hybrids were pollen sterile and could not be selfed, as expected for the progeny of wide crosses between species that differ in chromosome number (Eubanks, 1997). However, the hybrids could be used as female parent in backcrosses to either parent and produced seed from which plants were raised by ovule rescue on agar plates. The establishment of more advanced backcross populations should therefore be possible.

To generate backcross progeny, 40 to 50 flowers and flower buds of an F_1 hybrid were pollinated with pollen from *A. lyrata* or *A. thaliana*. The backcross to *A. lyrata* yielded 41 plants and the backcross to *A. thaliana* yielded 10 plants. As expected, plants in the first backcross (BC1) generations showed extremes in variability for a number of developmental traits. One example of this variability is provided by leaf form. As shown in Figure 5, the *A. thaliana* leaf blade is entire, whereas the *A. lyrata* leaf blade is lobed and is characterized by a large terminal segment with shallow lobes and a smaller basal segment with prominent lobes. The leaves of plants derived from backcrossing an F_1 hybrid to either the *A. lyrata* (Fig. 5) or *A. thaliana* parents varied both in size and morphology, and the leaf blades of individual plants differed significantly from one another and from the leaf blades of either parental species in extent of lobing and numbers of lobes.

The occurrence of such transgressive variation suggests that genome and/or chromosome recombination is taking place between the diverged *A. thaliana* and *A. lyrata* genomes. Recombination of genomes, resulting from independent assortment of complete chromosomes, either alone or in combination with crossing over between homeologous chromosomes or chromosomal segments, can theoretically produce a very large number of different chromosome constitutions and would account for the high degree of morphological variation we observed. The occurrence of genome recombination in the BC1 populations was verified by molecular methods, as illustrated by our analysis of plants generated in the backcross to *A. thaliana*. Simple sequence length polymorphisms (SSLPs) are often used for molecular mapping in Arabidopsis using Columbia (Col) × Landsberg *erecta* (Ler) populations (Lukowitz et al., 2000). To make use of existing SSLP markers and to follow the segregation of chromosome segments contributed by each parent, an interspecific F_1 hybrid was backcrossed to the Arabidopsis Ler strain rather than to the Col strain, which was used for the interspecific hybridization.

From an initial survey of 22 SSLP primer pairs, 17 primer pairs were found to be informative and to give reproducible results (Table I, see "Materials and Methods"). Ten backcross plants were examined with these SSLP markers. As shown in Table I, all plants contained Arabidopsis Ler-derived markers as expected, but they differed in the proportion of *A. lyrata*-derived markers they contained. However, for any set of markers that map to the same chromosome in *A. thaliana*, all informative markers were entirely derived from *A. thaliana* or entirely derived from *A.*

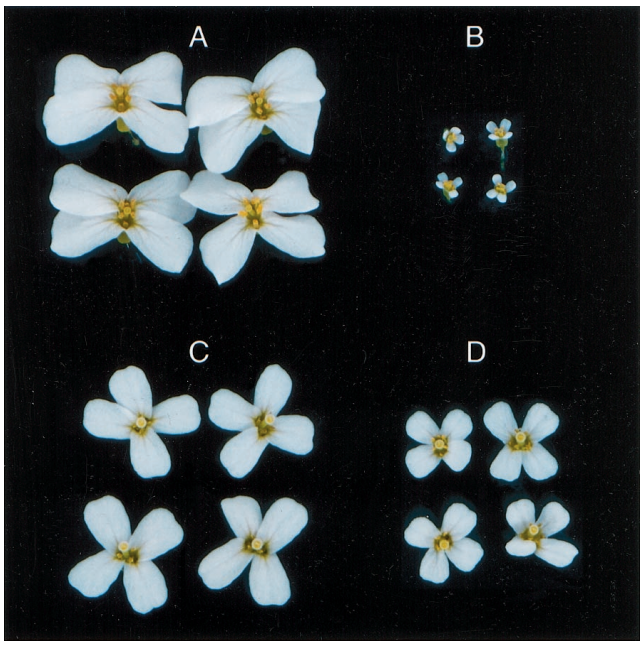

Figure 3. Mature flowers of *A. lyrata* (A), *A. thaliana* (B), their interspecific hybrid (C), and derived amphiploid (D). Images are approximately 2× actual size.

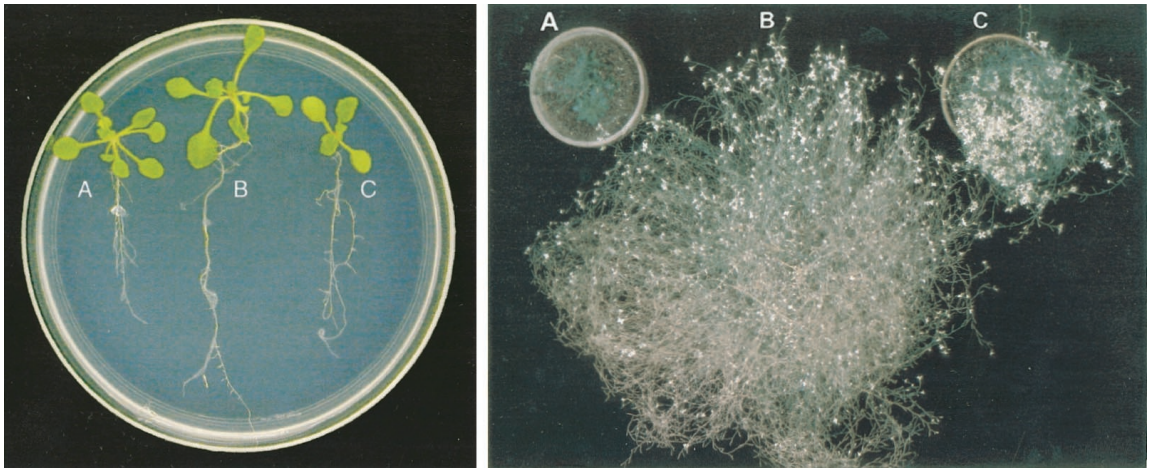

Figure 4. Hybrid vigor and luxuriance of the Arabidopsis interspecific hybrids in 3-week-old seedlings (left) and in mature plants (right). A, The *A. thaliana* parent; B, an interspecific hybrid; C, the *A. lyrata* parent. The seedlings were grown in a 10-cm Petri dish at 24°C and 16-h days. The three mature plants were grown in 15-cm pots.

lyrata in individual backcross plants. Thus, this initial analysis demonstrated the assortment of *A. lyrata* and *A. thaliana* chromosomes but did not detect crossing-over between homeologous segments of the *A. lyrata* and *A. thaliana* genomes.

A comparison of the progenies derived from backcrossing the F_1 hybrids to each of the two parental species provides yet another illustration of the varying outcomes of mixing diverged genomes. The F_1 hybrids all produced morphologically normal, albeit pollen-sterile, flowers. However, backcrossing one hybrid plant to each of the two parents resulted in dramatically different states of anther development. All 19 progenies from the backcross to the Arabidop-

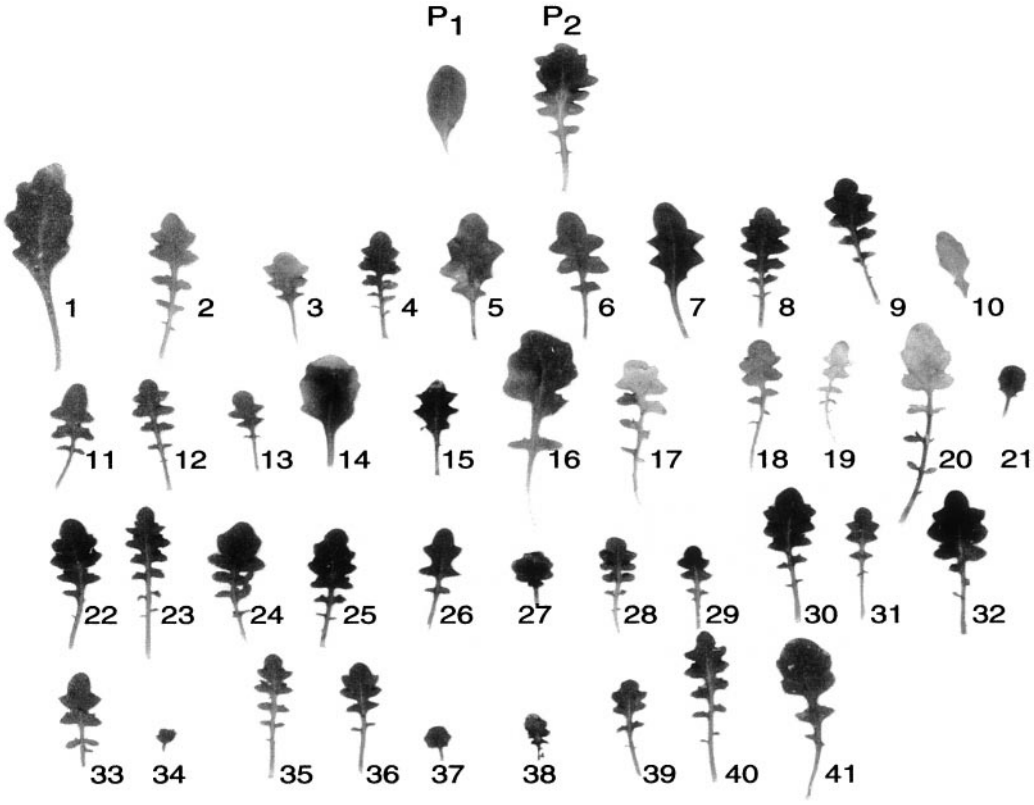

Figure 5. Transgressive variation in the leaf morphology of backcross plants. Mature leaves taken from fully developed rosettes are shown for the two parental species *A. lyrata* (P1) and *A. thaliana* (P2), and for 41 plants generated by backcrossing one interspecific F_1 hybrid plant to *A. lyrata*. Images are 0.5× actual size.

Table I. *SSLP analysis of the (Arabidopsis Col × A. lyrata) × Arabidopsis Ler backcross population*

Arabidopsis Chromosome	SSLP Marker (cM)[a]	Size (bp) of Amplified Fragment			Marker Assortment in Individual Backcross Progenies[b]				
		Col	Ler	*lyr*	Pattern 1	2	3	4	5
I	**F21M12** (10)	200	155[c]	160	Col	Col	Col	Col	*lyr*
	ciw12[d] (39)	128	~115	—[e]	Col	Col	Col	Col	*lyr*
	ciw1 (72)	165[c]	~143[c]	150	Col	Col	Col	Col	*lyr*
	nga280 (81)	105	85	~78	Col	Col	Col	Col	*lyr*
	nga111 (113)	128	162	~153	Col	Col	Col	Col	*lyr*
II	ciw2 (11)	105	~90	—[f]					
	ciw3 (30)	240[d]	210[c]	~275	Col	Col	Col	*lyr*	*lyr*
	nga1126 (50)	191	215[c]	~195					
	nga168 (73)	165[c]	135	105	Col	Col	Col	*lyr*	*lyr*
III	nga162 (20)	107	89	~80					
	ciw11[d] (43)	179	~230	230	Col	Col	*lyr*	*lyr*	*lyr*
	ciw4 (70)	190	~215	~180	Col	Col	*lyr*	*lyr*	*lyr*
	nga6[e] (86)	143	123	~125	Col	Col	*lyr*	*lyr*	*lyr*
IV	**ciw5** (10)	164	~144	~150	Col	*lyr*	*lyr*	*lyr*	*lyr*
	ciw6 (47)	162	~148	~150	Col	*lyr*	*lyr*	*lyr*	*lyr*
	ciw7 (65)	130	~123	~113	Col	*lyr*	*lyr*	*lyr*	*lyr*
	nga1107 (104)	150	140	~115	Col	*lyr*	*lyr*	*lyr*	*lyr*
V	CTR1 (10)	159	143	~155					
	ciw8 (42)	100	135	—[f]					
	PHYC (71)	207	222	~185	Col	Col	Col	*lyr*	*lyr*
	ciw9[d] (88)	165	~145	~145	Col	Col	Col	*lyr*	*lyr*
	ciw10[d] (115)	140	~130	~130	Col	Col	Col	*lyr*	*lyr*
Number of backcross progenies showing pattern					*2*	1	1	*1*	*5*

[a] SSLP markers, shown with their map position on *A. thaliana* chromosomes, were obtained from the supplemental material compiled by Lukowitz et al. (2000) found at http://carnegiedpb.stanford.edu/methods/ppsuppl.html. Markers that were used in our analysis are shown in bold characters (see "Materials and Methods"). [b] The Arabidopsis Ler-derived markers inherited yb each plant are not shown. [c] Sizes differ slightly from published sizes. [d] Markers for which genotype was inferred as described in "Materials and Methods." [e] The nga6 marker was previously reported not to amplify *A. lyrata* DNA (van Treuren et al., 1997). [f] No amplification.

sis Ler strain produced flowers containing sepals, petals, anthers, and carpel in the expected numbers (Fig. 6A). In contrast, only 2 of 42 progenies from the backcross to *A. lyrata* produced flowers bearing stamens with well-developed anthers. All other plants produced flowers in which stamens appeared as filament-like structures lacking normal anthers (Fig. 6, B and C). These third-whorl filaments varied between plants both in length and in the extent of anther development: they either lacked anthers (38 plants, Fig. 6B) or they terminated in an expanded structure that resembled a rudimentary anther (two plants, Fig. 6C). These structures resemble the third-whorl filamentous organs observed in several Arabidopsis mutants including *ufo* (Wilkinson and Haughn, 1995), *afo* (Kumaran et al., 1999), and *fil* (Sawa et al., 1999), and have also been observed in species hybrids (Michaelis, 1954; Malik et al., 1999; Matsuzuwa et al., 1999).

Spontaneous Generation of a *thaliana-lyrata* Amphidiploid

Among our five *thaliana-lyrata* sterile F_1 hybrids, we noticed that one plant produced a few fertile shoots (Fig. 7) 6 months into a continuous flowering period marked by the production of hundreds of shoots that lacked seed set. Seed from the self-fertile shoots of this hybrid plant produced fertile and vigorous plants that shared nearly identical growth habit and morphological characteristics, indicating that they all arose from the same event. This uniformity and vigor was carried over into two subsequent generations obtained by selfing the first-generation amphiploids. Cytological studies of plants in the first amphiploid generation (Fig. 1D) showed that these fertile plants contained 26 chromosomes (i.e. double that of the sterile hybrid plant from which they arose) and were therefore amphidiploid. Microscopic observation of pollen from dehisced anthers (Fig. 8) demonstrated the presence of fully developed grains that were approximately 1.5-fold larger than grains from the parental species (as expected for an amphiploid [Heslop-Harrison, 1998]) and of small malformed grains. Based on the proportion of these misshapen and presumably sterile grains, pollen viability in the amphiploid plants was estimated to range from 20% to 50%. This partial sterility may be due to the formation of multivalents between homeologous segments in the *A. thaliana* and *A. lyrata* genomes.

In general, the first-generation amphiploids and their progenies in two subsequent generations resem-

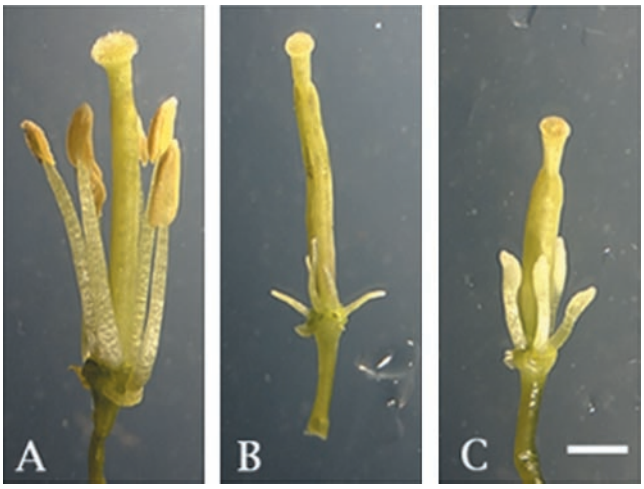

Figure 6. Anther development in plants generated by backcrossing one interspecific F_1 hybrid plant to the parental species. In the backcross to *A. thaliana*, all plants exhibited well-developed anthers (A). In the backcross to *A. lyrata*, the plants exhibited varying degrees of anther development with some plants having stamens reduced to filament-like structures lacking anthers (B) and other plants having stamens with rudimentary anthers (C). Bar = 1 mm.

bled their F_1 interspecific hybrid progenitor, although their more fleshy and robust leaves gave the appearance of more substantial rosettes. All amphiploids, however, produced smaller flowers than their F_1 hybrid parent with petal size approximately one-half that of the F_1 hybrid (Fig. 3). This result is unexpected because plants with increased ploidy are usually characterized by larger vegetative and reproductive structures relative to their diploid relatives, apparently because of increased cell size. However, epigenetic gene silencing events have been associated with increased ploidy levels in plants and are thought to "silence" the excessive alleles carried by the polyploid, thus providing a rapid means of "diploidizing" its genome (Mittelstein Scheid et al., 1996). It is possible that the smaller flower size in the amphiploids is one of the more conspicuous manifestations of such epigenetic gene silencing events or of some other gene dosage-compensation mechanism.

DISCUSSION

We have described some properties of the Arabidopsis species hybrids that we generated by crossing the diploid species *A. thaliana* and *A. lyrata.* The hybrids and backcross-1 progenies were pollen sterile, but they produced functional ovules, which should allow for the generation of more advanced backcross populations in which fertility is expected to be restored.

SSLP analysis of 10 plants derived from a backcross of an F_1 hybrid to *A. thaliana* provided evidence for independent assortment of *A. thaliana* and *A. lyrata* chromosomes. However, crossing-over events between homeologous segments of the two genomes were not detected, indicating that they occur at lower than normal frequency. Should further studies indicate that crossing-over between *A. thaliana* and *A. lyrata* chromosomes occurs at appreciable frequency, advanced backcross populations would allow a direct genetic analysis of the differences between the two parental species and the eventual positional cloning of the genes encoding these differences. This capability would be especially valuable for the study of the differences in mating system (inbreeding versus outbreeding) and growth habit (annual versus perennial).

In addition to basic physiological processes, several aspects of genome evolution may be investigated using the Arabidopsis species hybrids. For example, backcross populations of *thaliana-lyrata* hybrids may be used to investigate the extent of genetic differentiation between *A. thaliana* and *A. lyrata* and the degree to which it might interfere with gene flow between the two species (Rieseberg et al., 1996, 1999, 2000; Ungerer et al., 1998). A possible explanation for our observation that anther morphogenesis was severely impaired in the backcross to *A. lyrata*, but was relatively normal in the backcross to *A. thaliana*, is that the *A. thaliana* cytoplasm has diverged significantly since separation of the two species and has become more or less incompatible with nuclear genes from *A. lyrata.* Similar nucleo-cytoplasmic interactions were proposed to explain the drastically different outcomes of backcrossing *Epilobium* species hybrids to their parental species (Michaelis, 1954). The

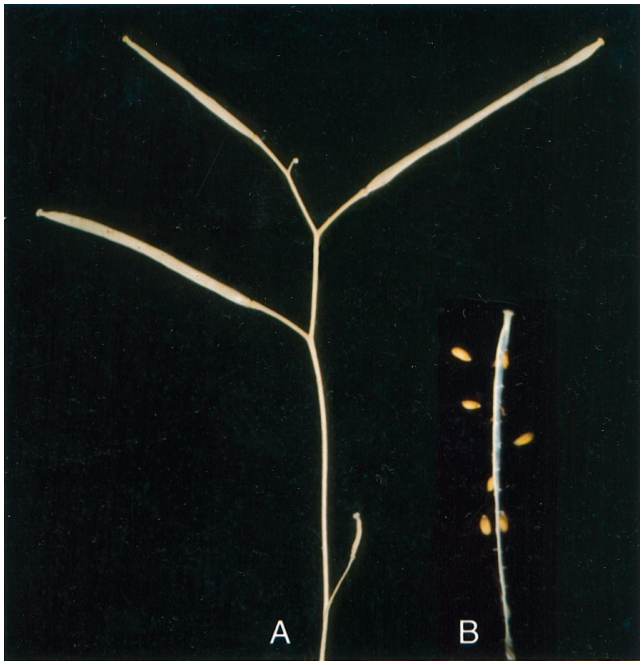

Figure 7. Spontaneous genesis of an amphiploid. A, One of the fertile inflorescences that were observed amid the many hundred inflorescences that developed on a long-lived sterile F_1 interspecific hybrid. B, One of the siliques containing several seeds that was produced on the fertile inflorescences.

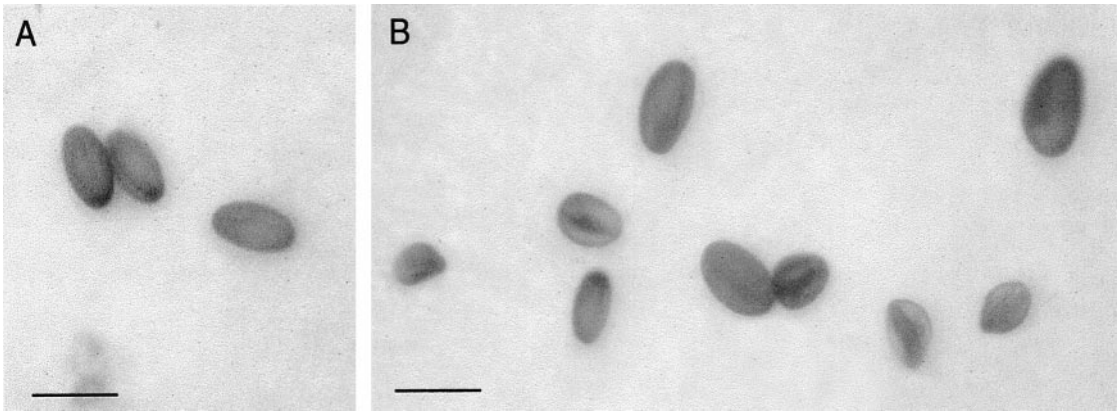

Figure 8. Pollen viability in the amphiploid generated by spontaneous chromosome doubling in an F_1 interspecific hybrid. A, Uniform appearance of dry pollen grains from the diploid *A. lyrata*. B, Heterogeneous appearance of dry pollen grains from the amphiploid. Note that 50% of the pollen grains are small, misshapen, and presumably sterile. The other 50% of the grains are fully developed and are larger in size than the pollen grains of the *A. lyrata* diploid strain. Bars = 40 μm.

aberrant stamen development observed in the majority of plants in the backcross to *A. lyrata* would reflect the imbalance created by an increased representation of the *A. lyrata* nuclear genome in the *A. thaliana*-derived cytoplasm. The two exceptional plants that produced anthers may not have inherited the specific complement of *A. lyrata* genes or chromosome segments that condition nucleo-cytoplasmic incompatibility. The two plants alternatively may have inherited specific *A. thaliana* genes that can override the negative nucleo-cytoplasmic interactions.

Studies of more advanced backcrosses might identify additional factors that contribute to genetic isolation between the two species. They might also identify chromosomal segments that are introgressed at high rates because they are positively selected and increase the fitness of backcross progeny (Rieseberg et al., 1999). The identification of the latter class of loci would provide a basis for the study of heterosis, a phenomenon that is poorly understood despite its perceived importance in evolution and its practical significance in breeding programs that aim to increase yield of crop plants.

The spontaneous generation of *thaliana-lyrata* amphidiploids provides yet another opportunity for investigating genome evolution and the interactions of divergent genomes. Polyploidy is quite common in plants and has played a major role in higher plant evolution (Clausen et al., 1945; Song et al., 1995; Leitch and Bennett, 1997; Liu et al., 1998; Soltis and Soltis, 1999; Wendel, 2000). In particular, amphiploidy, which involves the merger of two or more differentiated genomes has significant potential for species diversification. Amphiploids can arise as a result of the union of unreduced gametes or as a result of somatic chromosome doubling, both of which restore bivalent pairing, regular meiosis, and thus fertility. The spontaneous genesis of fertile amphiploid neospecies from sterile species hybrids has been observed in only a few instances, all reported early in the 20th century (Digby, 1912; Pellew and Durham, 1916; Clausen and Goodspeed, 1925; Karpechenko, 1927). The spontaneous appearance of an amphidiploid in one of our interspecific hybrids supports recent molecular studies demonstrating that amphiploidy occurs relatively frequently in plants with individual amphiploid species having originated independently and multiple times from the same diploid species (Soltis and Soltis, 1999). A likely explanation for the appearance of fertile siliques on an otherwise pollen-sterile *thaliana-lyrata* hybrid plant is that a somatic chromosome-doubling event occurred that led to the production of pollen in the shoot that gave rise to the amphidiploid. This event was in all probability favored by the long growth period of the perennial hybrid (Grant, 1981) and possibly by polysomaty (i.e. the occurrence within a plant of cells with different amounts of nDNA), which has been described in Arabidopsis (Galbraith et al., 1991; Mittelstein Scheid et al., 1996). Polysomaty, and presumably the differential endoploidy that produces it, are subject to developmental regulation and are influenced by environmental conditions (de Rocher et al., 1990; Smulders et al., 1994). Thus, it is possible that chromosome doubling in the sterile F_1 hybrid resulted from the normal occurrence of polysomaty in the cells of the shoot apical meristem that gave rise to the fertile shoots.

In any event, and in view of the availability of the Arabidopsis genome sequence, the *thaliana-lyrata* amphiploids will allow the analysis of genome evolution in polyploids at a level of detail not possible in other species. Furthermore, because these amphiploids originated from diploid parental species, their analysis should be more straightforward than that of *A. suecica* amphiploids, which are derived from tetraploid parents (Chen et al., 1998). The first-generation amphiploid plants were morphologically uniform, consistent with the conclusion that a single chromosome doubling event led to their production.

Analysis of subsequent amphiploid generations will determine if, as reported for other amphiploids (Song et al., 1995), these amphiploids will undergo rapid karyotypic and genomic changes (including elimination of specific sequences, chromosome segments, or entire chromosomes), as well as epigenetic changes (such as differential DNA methylation, gene-dosage compensation, gene silencing). It will be interesting to determine if these changes occur in a random fashion or in a non-random (and thus predictable) fashion in different amphidiploid plants and what impact these changes will have on developmental and physiological processes.

MATERIALS AND METHODS

Plant Material

Arabidopsis lyrata subsp. *lyrata* is a herbacious outcrossing perennial whose range in North America extends from Minnesota and Wisconsin south into Missouri, east into Georgia, north into Vermont, and west into Ontario (O'Kane and Al-Shehbaz, 1997). We used *A. lyrata* plants descended from accessions collected in Michigan (kindly provided by Charles Langley, University of California at Davis), and Arabidopsis ecotypes Col and Ler.

Ovule Rescue

The anthers of plants to be used as female parents in interspecific crosses and backcrosses were removed prior to anther dehiscence and stigmas were manually pollinated. Siliques were harvested 1 month after pollination. Ovules were dissected aseptically and placed on germination medium consisting of 4.33 g L^{-1} Murashige and Skoog salts, 10 g L^{-1} Suc, 100 mg L^{-1} myo-inositol, 1 μg L^{-1} thiamine, 0.5 μg L^{-1} pyridoxine, 0.5 μg L^{-1} nicotinic acid, 0.5 g L^{-1} MES [2-(*N*-morpholino)ethanesulfonic acid], and 0.8% (w/v) agar. Plates were incubated in a growth chamber at 25°C under continuous lighting until the emergence of plantlets, which were then transferred to soil.

Microscopic Analyses and Imaging

Examination of pollen tube development at the stigma surface was performed using UV-fluorescence microscopy as previously described (Kho and Baer, 1968). Chromosome counts were performed essentially as described by Heslop-Harrison (1998). Photomicrographs of chromosomes and dry pollen grains were taken using a Zeiss MC63 camera mounted on a Zeiss microscope and subsequently scanned for image analysis. Plants, flowers, and leaves were photographed with a Zeiss digital camera mounted on a stereoscope. Measurements were made using the NIH Image software package.

DNA Gel-Blot and SSLP Analysis

DNA was isolated from leaves according to Murray and Thompson (1980). DNA gel-blot analysis was performed as previously described (Conner et al., 1998). SSLP analysis (Bell and Ecker, 1994) was performed using the primers and strategy described in Lukowitz et al. (2000). An initial survey of 22 SSLP primer pairs identified 19 primer pairs that amplified *A. lyrata* DNA. Of these, 13 primer pairs produced a fragment that could be distinguished readily and reproducibly by agarose gel electrophoresis from the fragments amplified from Arabidopsis Ler and Col DNA (Table I) and thus allowed direct genotyping of the plants. In addition, one primer pair (ciw12) that did not amplify *A. lyrata* DNA and three primer pairs (ciw9, ciw10, ciw11) that did not identify a clear polymorphism between *A. lyrata* and Arabidopsis Ler were nevertheless informative because the genotype of individual plants could be inferred from the absence of the Arabidopsis Col-derived fragment.

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